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Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci

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Meta-Analysis Increases to 71 the Tally of Confirmed Crohn's Disease Susceptibility Loci

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We undertook a meta-analysis of six Crohn's disease (CD) genome-wide association studies (GWAS) comprising 6,333 cases and 15,056 controls, and followed up the top association signals in 15,694 cases, 14,026 controls and 414 parent/offspring trios. Thirty new susceptibility loci meeting genome-wide significance ($P\text{-value} < 5 \times 10^{-8}$) were identified. A series of *in silico* analyses highlighted particular genes within these loci and, together with manual curation, implicated functionally interesting candidate genes including *SMAD3*, *ERAP2*, *IL10*, *IL2RA*, *TYK2*, *FUT2*, *DNMT3a*, *DENND1B*, *BACH2* and *TAGAP*. Combined with previously confirmed loci, the results described here identify a total of 71 distinct loci with genome-wide significant evidence for association with Crohn's disease.

Crohn's disease (OMIM #266600) results from the interaction of environmental factors, including the intestinal microbiota, with host immune mechanisms in genetically susceptible individuals. Along with ulcerative colitis (UC), it is one of the main subphenotypes of inflammatory bowel disease (IBD). GWAS have highlighted key CD pathogenic mechanisms, including autophagy and Th17 pathways. A meta-analysis of these early scans implicated 32 susceptibility loci, but only accounted for 20% of the genetic contribution to disease risk - suggesting that more loci await discovery¹. Recognizing that an increased sample size would be required to detect these, we have expanded the International IBD Genetics Consortium (IIBDGC), approximately doubling the discovery panel size in comparison with the first meta-analysis.

The discovery panel for the current study comprised 6,333 CD subjects and 15,056 controls, all of European descent, with data derived from six index GWAS studies (for overview see Supplementary Table 1)²⁻⁶. Imputation using HapMap3 reference data allowed us to test for association at 953,241 autosomal SNPs. Our discovery panel had 80% power to detect variants conferring odds ratios 1.18 at the genome-wide significance level of $P < 5 \times 10^{-8}$, assuming a minor allele frequency 20% in healthy controls. Under the same conditions, the sample size of our original meta-analysis had only 11% power¹.

A quantile-quantile plot of the primary meta-statistic, using single-SNP Z-scores combined across all sample sets, showed a marked excess of significant associations (Supplementary Figure 1). A total of 2,024 SNPs within 107 distinct genomic loci, including all previously defined significant hits from our earlier meta-analysis, demonstrated association with $P\text{-values} < 10^{-5}$. A Manhattan plot is shown in Supplementary Figure 2. 51 of the regions, representing new loci associated at $P < 5 \times 10^{-6}$, were followed up by genotyping the most significant SNPs in an independent panel of 15,694 CD cases, 14,026 controls and 414 parent/offspring trios (see Table 1 and Supplementary Table 2).

Variants within 30 distinct new loci met a genome-wide significance threshold of $P < 5 \times 10^{-8}$ for association with CD in the combined discovery plus replication panel, with at least nominal association in the replication panel (see Table 1). Two additional loci, encompassing the *CARD9* and *IL18RAP* genes, had previously been reported as associated with CD in a candidate gene study⁷ and were here both replicated and confirmed at $P < 5 \times 10^{-8}$. Five loci were identified at genome-wide significance in GWAS studies

published subsequent to our replication experiment being designed. One, the FUT2 locus, was from a recent adult CD GWAS⁶. Four more (*ZMIZ1*, *IL27* at 16p11, 19q13 and 22q12) were identified in a pediatric IBD population⁵, these replicating here in our current sample set. Two further loci had produced “suggestive” evidence of association with replication in our earlier study¹. Here, these clearly exceeded the genome-wide significance threshold in the meta-analysis alone and, given the previous replication evidence, were not followed up further (see Table 1). Thus cumulatively, 39 additional loci can now be added to the 32 confirmed CD susceptibility loci identified at the time of the Barrett *et al.* study. We did not observe statistically significant heterogeneity of the odds ratios (Breslow Day test P-value <0.05 after Bonferroni correction; Supplementary Table 4) between the panels from our 15 different countries (Supplementary Tables 1 and 2) for any of the 71 loci. Nor was any evidence of interaction between the associated loci observed (Supplementary Figure 3).

Regional association plots of all 71 susceptibility loci including the underlying genes are shown in detail in Supplementary Figure 4, and complete genotype data including odds ratios and allele frequencies are shown in Supplementary Tables 3 and 4. Five loci had evidence for more than one independently associated variant (Table 1). While 6 of the 30 novel regions contain just a single gene, which is thereby strongly implicated in CD pathogenesis (e.g. *SMAD3*, *NDFIP1* and *BACH2*), 22 include more than one gene within the associated interval (Table 1; two regions without any gene or gene prediction). We thus applied additional *in silico* analyses to refine the list of functional candidate genes further. These were:

1. Interrogation of a publicly available expression quantitative trait loci (eQTL) database⁸. These analyses identified genes for which expression correlates with genotype at our most associated SNP (see Supplementary Results).
2. Use of 1000 Genomes Project Pilot sequence data and HapMap3 to identify genes containing non-synonymous variants in strong LD ($r^2 > 0.5$) with the focal SNP within each region (for details on coding SNP see Supplementary Table 5).
3. Use of GRAIL⁹, to identify non-random and evidence-based connectivity between the genes in the 71 confirmed CD loci. Specifically, GRAIL evaluates each gene in a CD-associated locus for non-random correlation with genes in the other 70 loci via word-usage in PubMed abstracts related to the gene (see Figure 1).

Summary results of these analyses are shown in the rightmost column of Table 1. The highlighted genes are described briefly in Box 1, as are genes that constitute particularly noteworthy candidates from intervals containing one or few genes. While we believe that these evidence-based approaches are helpful in identifying likely functional candidates, in some instances the different techniques highlight different genes. This reflects uncertainty as to which is causal, and highlights the need for functional studies.

30 new signals were identified here beyond those described in the earlier meta-analysis¹ and other subsequent publications. The new associations were driven primarily by increased power arising from the expanded sample size rather than improved imputation, as more than two-thirds of the novel loci identified here have good proxies ($r^2 > 0.8$) on both earlier generation arrays (Illumina 300K and Affymetrix 500k Set). Extending this argument beyond the current analysis, it seems likely that many more loci of modest effect size still await discovery.

For many of the novel loci, associations have been reported previously in other complex diseases, comprising mostly chronic inflammatory disorders (Table 1). Such diseases can cluster both within families and individuals, reflecting shared genetic risk factors. For example, IBD and ankylosing spondylitis can co-segregate and both are associated with

IL23R^{2,10} and *TNFSF15*^{11,12}. The *IL10* locus was previously associated with UC¹³ and was identified as a novel CD locus in the present study. Thus *IL10* is a generic IBD locus, which is a functionally intuitive finding of potential therapeutic significance.

For loci previously associated with other inflammatory diseases the direction of effect in CD is usually the same, but in five cases the risk allele for one disease appears to be protective in another disease (see arrow symbol in “Reported association” column in Table 1). In most such instances, functional annotation suggests modulation of T cell and other immune pathways. Indeed, GRAIL highlights a number of such genes. These inverse associations may reflect overlap in the pathways by which the host regulates effector functions in defense and regulatory functions in self-tolerance. This is a delicate balance and, in the face of competing requirements, selection pressures may have conferred advantage for divergent alleles in a cell- and environmentally dependent manner.

The associated SNP rs281379 at 19q13, recently also identified by McGovern *et al.*⁶ is highly correlated ($r^2 > 0.80$) with a common nonsense variant (rs601338 also known as G428A or W142X) at *FUT2*. This is classically referred to as the non-secretor variant, as individuals homozygous for this null-allele do not secrete blood group antigens at epithelial surfaces. Recently, non-secretors were identified as having near-complete protection from symptomatic GII.4 norovirus infection¹⁴ and the same null allele is identified here as a CD risk factor. This suggests one potential elusive link between infection and immune-mediated disease.

In contrast to the implication of coding variation in the *FUT2* gene, our previous data demonstrated that most CD-associated SNPs were not in LD with coding polymorphisms¹, suggesting that regulatory effects are likely to be a more common mechanism of disease susceptibility. Providing further direct evidence for this, a number of new eQTL effects were identified here (see Table 1 and Supplementary Results Section) – notably including *CARD9* (LOD=12.4), *ERAP2* (LOD=47.2) and *TNFSF11* (*RANKL*) (LOD=5.9). The latter maps adjacent to but outside the associated recombination interval, suggesting another potential long-range cis-regulatory effect as previously described for *PTGER4* in CD⁴. *RANKL* has pleiotropic immunological effects and also stimulates osteoclast activity. This finding may be relevant to the osteoporosis clinically associated with CD.

Given the importance of regulatory effects, it is intriguing that variants within the gene encoding a key mediator of epigenetic regulation, *DNA methyltransferase 3a* (*DNMT3A*), should be associated with CD. By inducing transcriptional silencing, DNMT3a is known to play an important role in immunoregulation. For example, it methylates *IL-4* and *IFN- γ* promoters following T cell receptor stimulation, hence regulating T cell polarization¹⁵, and induces dynamic regulation of *TNF- α* transcription following lipopolysaccharide exposure in leukocytes¹⁶. Genetically determined alterations in DNMT3a activity could thus have far-reaching effects.

The 32 loci described up to 2008 explained approximately 20% of CD heritability. Adding the 39 loci described since increases the proportion of heritability explained to just 23.2%. This pattern of common alleles, explaining a logarithmically decreasing fraction of heritability (Figure 2), is consistent with a recent model of effect size distribution¹⁷, which predicted (based on the previous CD meta-analysis) that our current sample size would likely identify 48 new loci. Furthermore, it is likely that more high-frequency CD risk alleles of even smaller effect size remain unidentified: The same model predicts that 140 loci would be identified by a sample size of 50,000, but these would explain only a few more percent of CD heritability. It is clear, therefore, that larger GWAS alone will not explain all of the missing heritability in CD.

One key shortcoming of our current model of heritability explained by these loci is a direct consequence of the extent to which GWAS tag SNPs are often imperfect proxies for causal alleles, and thus substantially underestimate the true attributable risk. For example, the best tag SNP at the *NOD2* locus in our meta-analysis appears to explain just 0.8% of genetic variance, whereas the three *NOD2* coding mutations themselves account for 5%. If an analogous situation applies to even a small fraction of the other 70 CD susceptibility loci, the proportion of overall heritability explained will increase significantly. Indeed, one study of LD between tag SNPs and causal variants in the heritability of human height¹⁸ suggests that this effect might double the total fraction of heritability explained by GWAS SNPs. Coding variants identified here from the 1000 Genomes Project which are in strong LD with the focal SNPs in several of our regions (see Supplementary Table 4) thus now require direct assessment in order to explore this possibility.

Other factors will also account for the heritability gap, including uncertain epidemiological estimates of disease prevalence and total heritability, as well as our observation that several of the new regions contain more than one independent risk allele. The likelihood is that many more such effects will be identified. Indeed, detailed future analyses will play a key role in helping us to understand the absolute contribution of common causal alleles, as well as identifying less common variants and rare (even family-specific) mutations. By contrast, our lack of evidence for epistasis among the loci described here suggests that non-additive interactions among common risk alleles do not play an important role in the genetic architecture of CD.

The current study has approximately doubled the number of confirmed CD susceptibility loci. For many of these loci we have identified potentially causal genes, accepting that confirmation of their role must await detailed fine mapping, expression and functional studies. While the alleles detected only modestly affect disease risk, they continue to enhance our understanding of the genetic etiology of CD. Analysis for evidence of sub-phenotype associations represents an important future goal for the consortium. Thus, we are working towards sharing of detailed genotype and clinical data to allow this. In the meantime, extensive resequencing, together with large-scale fine mapping exercises using custom array-based technologies, are already underway and will further elucidate the pathogenic mechanisms of IBD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Box 1

Noteworthy genes within loci newly implicated in Crohn's disease pathogenesis. N.B. Although we highlight these as interesting genes, we do not yet have data to confirm causality

- **VAMP3** (1p36) encodes vesicle-associated membrane protein 3: following bacterial stimulation of TNF- α production within macrophages, VAMP3 interacts with SNARE proteins first on the trans-Golgi network, where TNF- α is taken up, and then on the cell membrane, where TNF- α is released¹⁹. VAMP3 also plays a role in cell migration and adhesion, by trafficking molecules such as beta1 integrin to the cell surface, and has been implicated in autophagy²⁰.
- **MUC1/SCAMP3** (1q22) *MUC1* encodes a key constituent of mucus, which is the physical barrier that protects the intestinal epithelium from gut bacteria. *MUC1* overexpression and hypoglycosylation have been reported in IBD²¹ and *Muc1* knockout mice exhibit increased small intestinal damage after *C. jejuni* infection²². Secretory carrier membrane protein 3 (*SCAMP3*) regulates EGFR trafficking within endosomal membranes²³. It is manipulated by intracellular salmonellae to acquire nutrients and influence host immune responses²⁴.
- **DENND1B** (1q31) has been recently associated with asthma²⁵, and is expressed in dendritic and effector memory T cells. Among other functions it modulates inflammatory signaling pathways, including Th1-Th2 cytokines, through repression of TNFR1 signaling.
- **IL10** (1q32): association with CD follows its recent implication in UC¹³ and reporting of mutations in the *IL10* receptors in extreme CD of infancy²⁶. Known to inhibit synthesis of pro-inflammatory cytokines within macrophages and Th1 cells, IL10 also suppresses antigen presenting cell activity. Knockdown of IL10 in mice presents one of the best animal models of IBD.
- **DNMT3A** (2p23): DNA methyltransferase 3a is one of three key methyltransferase genes in humans - effecting epigenetic regulation of gene transcription by methylating cytosine residues within CpG islands. Among many other roles this is known to determine dynamic regulation of both adaptive and innate immune mechanisms^{15,16}.
- **GCKR** (2p23) encodes an inhibitor of glucokinase, with the focal SNPs at this locus also correlating with both fibrinogen and CRP levels²⁷.
- **THADA** (2p21) is expressed in small intestine and appears to encode a death receptor-interacting protein suggesting an apoptotic function²⁸.
- **ERAP2** (5q15): regulated by NF- κ B, this gene encodes one of two human endoplasmic reticulum aminopeptidases, which work in concert to trim peptides for presentation on MHC class I and hence critically affect antigen presentation to T cells²⁹. Ankylosing spondylitis is associated with this locus but with a different pattern of associated variants³⁰. Given the close clinical relationship between CD and AS, and the strong association of HLA-B27 with the latter but not the former, the divergent association of these closely related molecules is intriguing and will refocus interest on the MHC class I associations in CD.
- **NDFIPL1** (5q31): Nedd4 family interacting protein 1 is a membrane protein involved in maintenance of the Golgi complex³¹. It is important for protein trafficking via exosomes and may play a role in rapid sequestration and removal of proteins during stress³².

- **CPEB4** (5q35) is a regulator of protein translation and cell division, and a transcriptional target of ROR γ t. Mouse work suggests that the product of *CPEB4* is the effector by which ROR γ t (a key determinant of Th17 cell differentiation) inhibits proliferation of thymocytes³³.
- **TAGAP** (6q25): T-cell Activation GTPase-Activating Protein, associated with multiple autoimmune diseases, was originally identified through its involvement in human T cell activation and co-regulation with IL-2³⁴.
- **IL2RA** (10p15) encodes part of the IL2 receptor complex, thus mediating IL2 signalling in host defence and regulating response to autoantigens by Tregs. The associated variants correlate with differential expression of IL2RA (CD25) on CD4+ naïve and memory T cells³⁵ possibly affecting Foxp3⁺ Treg homeostasis³⁶.
- **FADS 2** (11q12): fatty acid desaturase 2 is predominantly located in the endoplasmic reticulum. *Fads2* knockout mice develop duodenal and ileocecal ulceration³⁷.
- **TNFSF11** (13q14) (synonyms RANKL - receptor activator of nuclear factor kappa B; ODF osteoclast differentiation factor) encodes a member of the TNF cytokine family. RANKL stimulation of dendritic cells leads to proliferation of naive T cells and inducible Tregs³⁸; it also regulates osteoclast activity / bone loss. Previous studies have demonstrated increased plasma levels in Crohn's disease³⁹.
- **SMAD3** (15q22): phosphorylated following TGF- β signalling through its receptor, the SMAD3 protein complexes with SMAD4 and is then translocated to the nucleus to modulate target gene expression. SMAD3 plays a key role in the TGF- β -mediated induction of Foxp3⁺ regulatory T cells⁴⁰, with SMAD3 deficiency reciprocally enhancing Th17. Reduced SMAD3 phosphorylation has been observed in IBD, and may impair the immunosuppressive effect of TGF- β .
- **TYK2** (19p13) encodes tyrosine kinase 2, a member of the JAK-signal transduction family. It is involved in cytokine signaling by IFN- γ , IL-12 and IL-23 among others – hence affecting Th1 and Th17 lineage development. TYK2 also plays an important role in TLR-mediated responses in dendritic cells, including IL-12 and IL-23 production, and TYK2 mutations predispose to opportunistic infection⁴¹.
- **FUT2** (19q13) encodes alpha-(1,2)fucosyltransferase which regulates expression of the Lewis AB0(H) histo-blood group antigens on the surface of epithelial cells and in body fluids. Strongly associated with Norovirus infection, also with *Helicobacter pylori* infection and serum vitamin B12 levels^{14,42}.

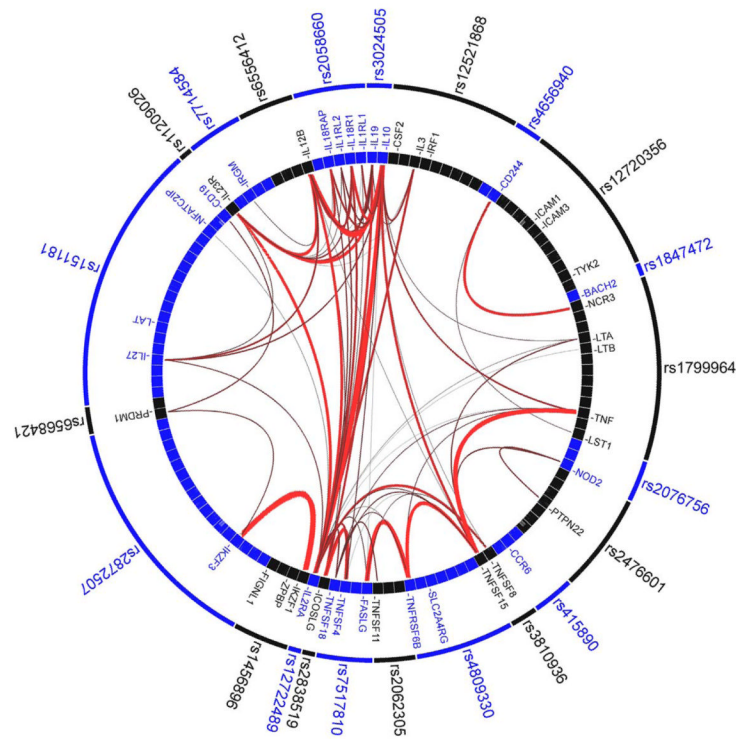


Figure 1. Gene Relationships Across Implicated Loci (GRAIL) pathway analysis

Links between genes at 23 of 71 Crohn's disease associated loci which scored $P < 0.01$ using GRAIL. Specifically, of the 71 CD-associated SNPs, 69 are in LD intervals containing or within 50 kb of at least one gene. In total, there are 355 genes implicated by proximity to these 69 SNPs. Each observed CD-association was scored with GRAIL, which takes all genes mapping within CD-associated intervals and evaluates for each whether it is non-randomly linked to the other genes, via word-usage in PubMed abstracts. 23 SNPs shown in the outer circle are $P < 0.01$ hits - indicating that the regions which they tag contain genes which are more significantly linked to genes in the other 68 regions than expected by chance at that level. The lines between genes represent individually significant connections that contribute to the positive signal, with thickness of lines inversely proportional to the probability a literature-based connection would be seen by chance.

To accurately assess the statistical significance of this set of connections, we conducted simulations where we selected 1000 sets of 69 SNPs implicating in total 355 ± 18 (5%) (selecting the SNPs randomly and using rejection sampling - only taking lists that implicated the same number of genes). Each of those 1000 sets were scored with GRAIL. The mean number of $P < 0.01$ hits in a simulated list was 0.91 with a range in the 1000 sets from 0 to 11, suggesting that the likelihood of observing 23 hits with $P < 0.01$ is far less than 0.1%.

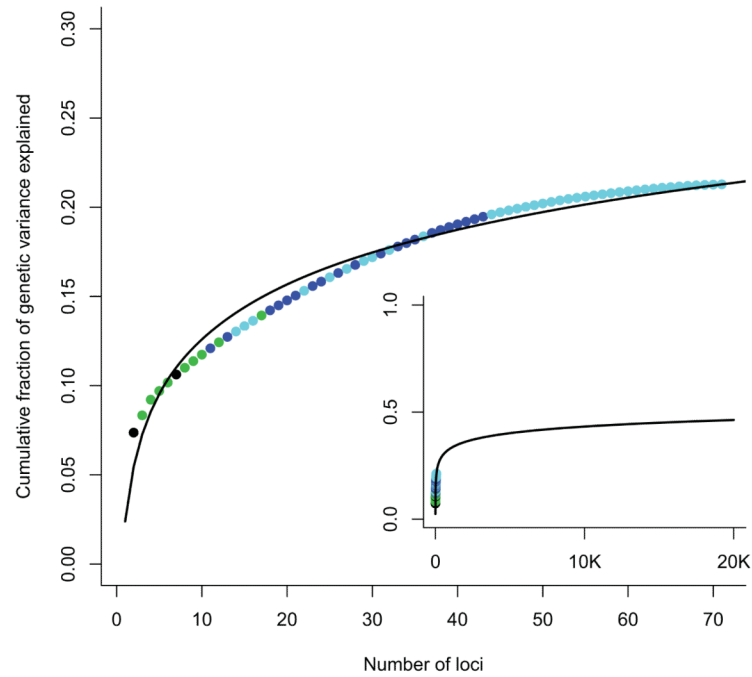


Figure 2. Cumulative fraction of genetic variance explained by 71 CD loci

Cumulative fraction of genetic variance explained by the 71 CD loci reported here, ordered from largest to smallest individual contribution. **Black** points were identified pre-GWAS, green in first generation GWAS, blue in an earlier meta-analysis and cyan in this analysis. Inset shows a logarithmic fit to these data extrapolated to an extreme scenario where 20,000 independent common alleles are associated with disease. Even in this situation less than half of the genetic variance would be explained. This demonstrates that other types of effect (e.g. less common and rare alleles with higher penetrance) must also exist.

Table 1
Association results and *in silico* analyses for all 71 confirmed CD loci

The upper tier lists new Crohn's disease susceptibility loci (beyond the first international meta-analysis¹) confirmed in the current study with a genome-wide significant P-value ($P < 5 \times 10^{-8}$) in the combined analysis (discovery + replication sample.) and $P < 0.05$ on replication. Results for replication are listed for all 39 loci which at the time of study design had not met $P < 5 \times 10^{-8}$ plus at least nominal evidence of replication in an independent sample set. The 7 loci identified in subsequent studies are identified (see footnotes). The lower tier lists new data for SNPs/loci confirmed in the earlier meta-analysis¹. Genomic positions were retrieved from NCBI's dbSNP build v130. Linkage disequilibrium (LD) regions around focal SNPs were defined by extending the region to the left for 0.1 cM or until another SNP with $P < 10^{-5}$ was reached, in which case the process was repeated from this SNP. Right-hand boundaries were defined in the same way. We identified loci previously associated with other relevant traits by a manual literature search and using the NIH catalog of published Genome-wide association studies and the HuGe database (version 1.4) (accessed on May 28th 2010) 43,44.

UC – ulcerative colitis, AS – ankylosing spondylitis, Ps – psoriasis, PBC – primary biliary cirrhosis, T1D – type 1 diabetes, RA – rheumatoid arthritis, SLE – systemic lupus erythematosus, celiac – celiac disease, T2D – type 2 diabetes, MS – multiple sclerosis, Graves – Graves disease, AD – Alzheimer's disease, MCV – mean corpuscular volume, ALL – acute lymphocytic leukemia, Lepr. – leprosy, SpA – spondyloarthritis, PD – Parkinson's disease, CRC – colorectal cancer, CRP – C-reactive protein, TGs – triglycerides, PC – prostate cancer, HSV – human simplex virus, CAD – coronary artery disease, CLL – chronic lymphocytic leukemia, BD – bone density, B12 – serum vitamin B12 levels, HP – *Helicobacter pylori*, AA – alopecia areata, AITD – autoimmune thyroid disease, BC – breast cancer, BD – Behcet's disease, GC – gastric cancer, Hep.C – hepatitis C susceptibility, SSC – systemic sclerosis, Myelo. – myeloproliferative disease, TB – tuberculosis, GvHD – Graft versus host disease, WBC – white blood cell count, HIES – hyper immunoglobulin E syndrome. Regional association plots for all 71 loci are shown in Supplementary Figure 4 and genotype data is shown in Supplementary Tables 3 and 4.

No.	dbSNP ID	Chr.	Left - right (Mb)	Risk allele - Allele frequency in control population	P-value meta	P-value repl.	P comb.	OR (95% CI); *Loci with evidence of > than 1 independent association	Reported association	Positional candidate genes of interest; some additionally highlighted by ▲ IKG cSNP(s) in LD; GRAIL (bold); eQTL (LOD score)
(a) New Loci meeting Genome-wide significance ($P\text{-value} < 5.0 \times 10^{-8}$) in this study										
1	rs2797685	1p36	7.66 - 7.89	A - 0.190	2.69×10^{-10}	1.40×10^{-2}	7.10×10^{-9}	1.05 (1.01-1.10)	Celiac	<i>VAMP3</i>
2	rs3180018	1q22	153.24 - 154.39	A - 0.250	1.29×10^{-9}	2.70×10^{-5}	2.30×10^{-13}	1.13 (1.06-1.19)*	T2D, Ashma, PD	<i>SCAMP3</i> , <i>MUC1</i>
3	rs1998598	1q31	195.58 - 196.21	G - 0.302	4.90×10^{-9}	1.60×10^{-2}	8.70×10^{-9}	1.04 (1.00-1.09)	Asthma	<i>DEVD1B</i>
4	rs3024505	1q32	204.87 - 205.10	T - 0.157	8.32×10^{-9}	1.50×10^{-7}	1.60×10^{-14}	1.12 (1.07-1.17)	T1D, UC, SLE, BD, Hep. C,	<i>IL10</i> , <i>IL19</i>
5	rs13428812	2p23	25.30 - 25.46	G - 0.326	1.41×10^{-8}	5.90×10^{-4}	8.50×10^{-10}	1.06 (1.03-1.10)		<i>DNMT3A</i>
6	rs780093	2p23	27.24-27.71	T - 0.418	1.10×10^{-4}	3.30×10^{-8}	4.70×10^{-11}	1.15 (1.10-1.21)	CRP, Glucose, TGs	▲ <i>GCKR</i>
7	rs10495903	2p21	43.30 - 43.80	T - 0.129	7.70×10^{-8}	2.90×10^{-8}	1.60×10^{-14}	1.14 (1.09-1.20)*	T2D, PC	▲ <i>THADA</i>
8 [§]	rs10181042	2p16	60.77 - 61.74	T - 0.420	6.61×10^{-9}	N/A	N/A	1.14 (1.09-1.19)	RA, UC, Celiac	<i>C2orf74 (9.6)</i> , <i>REL</i>
9 [†]	rs2058660	2q12	102.17 - 102.67	G - 0.231	1.58×10^{-12}	N/A	N/A	1.19 (1.14-1.26)	Celiac, Ashma, T1D, HSV	<i>IL18RAP</i> , <i>IL12RL2</i> , <i>IL18RI</i> , <i>IL1RL1</i>
10	rs6738825	2q33	197.85 - 198.67	A - 0.473	1.82×10^{-7}	1.60×10^{-3}	3.50×10^{-9}	1.06 (1.02-1.11)	CAD	▲ <i>PLCL1</i>

No.	dbSNP ID	Chr.	Left - right (Mb)	Risk allele - Allele frequency in control population	P-value meta	P-value repl.	P comb.	OR (95% CI); *Loci with evidence of > than 1 independent association	Reported association	Positional candidate genes of interest; some additionally highlighted by ▲ IKG cSNP(s) in LD; GRAIL (bold) ; eQTL (LOD score)
11	rs7423615	2q37	230.76 - 230.94	T - 0.187	4.57×10 ⁻⁹	7.40×10 ⁻⁶	3.10×10 ⁻¹³	1.12 (1.07-1.18)	CLL	<i>SP140</i> (8.8)
12	rs13073817	3p24	18.58 - 18.86	A - 0.322	8.20×10 ⁻⁷	1.00×10 ⁻³	6.70×10 ⁻⁹	1.08 (1.03-1.13)		
13	rs7702331	5q13	72.49 - 72.62	A - 0.600	2.00×10 ⁻⁶	6.40×10 ⁻⁷	5.90×10 ⁻¹²	1.12 (1.07-1.17)		<i>TMEM174</i>
14	rs2549794	5q15	96.11 - 96.45	C - 0.409	4.47×10 ⁻¹¹	2.00×10 ⁻³	1.10×10 ⁻¹⁰	1.05 (1.02-1.09)	AS, PD, T1D	▲ <i>ERAP2</i> , <i>LRAP</i> (47.2)
15	rs11167764	5q31	141.39 - 141.62	C - 0.796	1.10×10 ⁻⁹	4.20×10 ⁻³	2.00×10 ⁻⁹	1.06 (1.02-1.11)		<i>NDPFI</i>
16	rs359457	5q35	173.15 - 173.47	T - 0.571	5.25×10 ⁻⁸	3.30×10 ⁻⁶	2.50×10 ⁻¹²	1.08 (1.04-1.12)		<i>CPEB4</i> (6.1)
17	rs17309827	6p25	3.35 - 3.41	T - 0.639	6.16×10 ⁻⁷	3.10×10 ⁻⁴	6.70×10 ⁻⁹	1.10 (1.05-1.16)		<i>C6orf85</i>
18	rs1847472	6q15	90.86 - 91.14	G - 0.658	3.63×10 ⁻⁶	1.40×10 ⁻⁴	5.10×10 ⁻⁹	1.07 (1.03-1.11)	T1D, Celiac	<i>BACH2</i>
19	rs212388	6q25	159.26 - 159.46	G - 0.393	1.41×10 ⁻⁷	2.40×10 ⁻⁵	2.30×10 ⁻¹¹	1.10 (1.05-1.14)	RA, Celiac, T1D ↑	<i>TAGAP</i>
20	rs6651252	8q24	129.56 - 129.67	T - 0.865	2.29×10 ⁻⁶	2.40×10 ⁻¹³	3.90×10 ⁻¹⁸	1.23 (1.17-1.30)		
21 [†]	rs4077515	9q34	138.27 - 138.54	T - 0.411	4.37×10 ⁻¹⁹	1.50×10 ⁻¹⁹	1.30×10 ⁻³⁶	1.18 (1.13-1.22)	UC, AS	<i>CARD9</i> (12.4), ▲ <i>CARD9</i> , ▲ <i>SNAPC4</i>
22	rs12722489	10p15	6.07 - 6.21	C - 0.852	8.51×10 ⁻⁶	5.20×10 ⁻⁵	2.90×10 ⁻⁹	1.11 (1.05-1.16)	MS, T1D, Vitiligo, RA, AA, Asthma, AITD	<i>IL2RA</i>
23	rs1819658	10q21	59.50 - 59.81	C - 0.774	1.41×10 ⁻⁷	1.10×10 ⁻¹⁰	9.10×10 ⁻¹⁷	1.19 (1.13-1.25)	AD	<i>UBE2D1</i>
24 [‡]	rs1250550	10q22	80.67 - 80.77	G - 0.669	2.00×10 ⁻¹⁰	7.30×10 ⁻²²	1.10×10 ⁻³⁰	1.19 (1.15-1.23)	Celiac, MS, Vitiligo, BC	<i>ZMIZ1</i>
25	rs102275	11q12	61.28 - 61.44	C - 0.341	7.24×10 ⁻⁸	1.70×10 ⁻⁵	2.30×10 ⁻¹¹	1.08 (1.04-1.12)	CAD; Dyslipidemia	<i>FADS1</i> (5.0)
26	rs694739	11q13	63.58 - 64.05	A - 0.626	3.38×10 ⁻⁷	3.50×10 ⁻⁴	6.00×10 ⁻¹⁰	1.10 (1.05-1.16)	AA	<i>PRDX3</i> , <i>ESRRA</i>
27	rs2062305	13q14	41.72 - 42.00	G - 0.346	2.00×10 ⁻⁶	5.70×10 ⁻⁵	4.90×10 ⁻¹⁰	1.10 (1.05-1.15)	BD, RA	<i>TNFSF11</i> , <i>TNFSF11</i> (5.9)
28	rs4902642	14q24	68.23 - 68.39	G - 0.584	2.00×10 ⁻⁷	4.50×10 ⁻⁵	1.60×10 ⁻¹⁰	1.07 (1.11-1.04)*	Celiac, T1D	<i>ZFP36L1</i>
29	rs8005161	14q35	87.28 - 87.71	T - 0.119	1.29×10 ⁻⁸	5.90×10 ⁻¹¹	4.20×10 ⁻¹⁸	1.23 (1.16-1.31)*		▲ <i>GALC</i> , ▲ <i>GPR65</i> , <i>GPR65</i>
30	rs17293632	15q22	65.20 - 65.27	T - 0.233	1.41×10 ⁻¹³	2.00×10 ⁻⁸	2.70×10 ⁻¹⁹	1.12 (1.07-1.16)	CAD, T2D	<i>SMAD3</i>
31 [‡]	rs151181	16p11	28.20 - 28.94	G - 0.386	1.10×10 ⁻¹⁰	1.20×10 ⁻³	1.50×10 ⁻¹¹	1.07 (1.03-1.12)	T1D, obesity, Asthma, CRC, SLE, RA	▲ <i>APOB48R</i> , ▲ <i>IL27</i> , ▲ <i>SULT1A2</i> , ▲ <i>SULT1A1</i> , ▲ <i>SH2B1</i> , <i>EHFEC</i> (11.3), <i>IL27</i> , <i>LAT</i> , <i>CD19</i> , <i>NEFATC2IP</i>
32 [§]	rs3091315	17q12	29.51 - 29.70	A - 0.723	1.70×10 ⁻¹³	N/A	N/A	1.20 (1.14-1.26)	HIV resistance	<i>CCL2</i> , <i>CCL7</i>
33	rs12720356	19p13	10.26 - 10.50	G - 0.084	9.20×10 ⁻¹⁰	1.90×10 ⁻⁵	1.40×10 ⁻¹²	1.12 (1.06-1.19)*	T1D, SLE, MS, HIES	▲ <i>TYK2</i> , <i>TYK2</i> , <i>ICAM1</i> , <i>ICAM3</i>
34 [‡]	rs736289	19q13	38.42 - 38.47	T - 0.612	2.69×10 ⁻⁷	2.00×10 ⁻³	8.70×10 ⁻⁹	1.06 (1.02-1.11)		
35 [‡]	rs281379	19q13	53.78 - 53.97	A - 0.487	8.60×10 ⁻¹⁰	5.20×10 ⁻⁵	7.40×10 ⁻¹²	1.07 (1.04-1.11)	B12, Norovirus, HP	▲ <i>FUT2</i> , ▲ <i>RASIP1</i>
36	rs4809330	20q13	61.65 - 61.95	G - 0.709	2.51×10 ⁻¹²	4.60×10 ⁻⁵	2.70×10 ⁻¹⁵	1.12 (1.06-1.18)	Glioma	▲ <i>RTEL1</i> , <i>TNFRSF6B</i> , <i>SLC2A4RG</i>

No.	dbSNP ID	Chr.	Left - right (Mb)	Risk allele - Allele frequency in control population	P-value meta	P-value repl.	P comb.	OR (95% CI); *Loci with evidence of > than 1 independent association	Reported association	Positional candidate genes of interest; some additionally highlighted by ▲ 1KG cSNP(s) in LD; GRAIL (bold) ; eQTL (LOD score)
37	rs181359	22q11	20.14 - 20.39	T - 0.203	6.31×10 ⁻¹³	2.30×10 ⁻⁶	4.80×10 ⁻¹⁶	1.10 (1.06-1.15)	RA, Celiac, SLE, MCV	▲ <i>YDIC</i>
38 [‡]	rs713875	22q12	28.23 - 29.00	C - 0.471	5.70×10 ⁻⁹	8.30×10 ⁻⁵	7.30×10 ⁻¹²	1.08 (1.04-1.13)	T1D	<i>MTMR3</i>
39	rs2413583	22q13	38.00 - 38.14	C - 0.830	1.70×10 ⁻¹⁰	9.50×10 ⁻¹⁸	1.10×10 ⁻²⁶	1.23 (1.17-1.29)		<i>MAP3K7IP1</i>
(b) Loci that met genome-wide significance (P-value < 5.0×10 ⁻⁸) in Barrett et al.										
1	rs11209026	1p31	67.13 - 67.54	G - 0.932	1.00×10 ⁻⁶⁴	N/A	N/A	2.66 (2.36-3.00)	UC, AS, Ps, PBC, GC, BD	▲ <i>IL23R</i> , <i>IL23R</i>
2	rs2476601	1p13	113.66 - 114.42	G - 0.907	4.47×10 ⁻⁹	N/A	N/A	1.26 (1.17-1.37)	T1D [‡] , RA, SLE, Ps, Vitiligo [‡] , AITD	▲ <i>PTPN22</i> , <i>PTPN22</i>
3	rs4656940	1q23	158.96 - 159.20	A - 0.801	6.17×10 ⁻⁷	N/A	N/A	1.15 (1.09-1.21)	SLE, RA	<i>CD244</i> , <i>CD244</i> , <i>ITLN1</i>
4	rs7517810	1q24	170.92 - 171.21	T - 0.246	1.51×10 ⁻¹⁵	N/A	N/A	1.22 (1.16-1.28)	HepC, SLE, SSC, T2D	<i>TNFSF18</i> , <i>TNFSF4</i> , <i>FASLG</i>
5	rs7554511	1q32	199.11 - 199.32	C - 0.726	1.58×10 ⁻⁷	N/A	N/A	1.14 (1.08-1.19)	UC, celiac, MS	▲ <i>C1orf106</i> , <i>KIF21B</i>
6	rs3792109	2q37	233.81 - 234.23	A - 0.529	6.76×10 ⁻⁴¹	N/A	N/A	1.34 (1.29-1.40)	UC	▲ <i>ATG16L1</i>
7	rs3197999	3p21	48.16 - 51.73	A - 0.297	6.17×10 ⁻¹⁷	N/A	N/A	1.22 (1.16-1.27)	UC	▲ <i>MST1</i> , ▲ <i>GPX1</i> , ▲ <i>BSN</i>
8	rs11742570	5p13	39.88 - 41.00	C - 0.606	7.08×10 ⁻³⁶	N/A	N/A	1.33 (1.27-1.39)	MS	<i>PTGER4</i>
9	rs12521868	5q31	129.41 - 132.05	T - 0.422	1.41×10 ⁻²⁰	N/A	N/A	1.23 (1.18-1.28)	Ps, Fibrominogen, Asthma, TB, UC	▲ <i>SLC22A4</i> , <i>SLC22A5</i> (<i>5-4</i>), <i>IRF1</i> , <i>CSF2</i> , <i>IL3</i>
10	rs7714584	5q33	150.01 - 150.38	G - 0.088	7.76×10 ⁻¹⁹	N/A	N/A	1.37 (1.28-1.47)	TB	<i>IRGM</i>
11	rs6556412	5q33	158.43 - 158.88	A - 0.332	5.37×10 ⁻¹⁴	N/A	N/A	1.18 (1.13-1.24)	Ps, SLE, Malaria, Asthma	<i>IL12B</i>
12	rs6908425	6p22	20.60 - 21.25	C - 0.784	1.41×10 ⁻⁸	N/A	N/A	1.17 (1.11-1.23)	T2D, Ps, UC	<i>CDKAL1</i>
13	rs1799964	6p21	31.49 - 32.98	C - 0.209	3.98×10 ⁻¹¹	N/A	N/A	1.19 (1.13-1.25)	Multiple including UC	▲ <i>MCCDL</i> , ▲ <i>LTA</i> , <i>HLA-DQA2</i> , <i>TNF</i> , <i>LST1</i> , <i>LTA</i> , <i>NCR3</i>
14	rs6568421	6q21	106.50 - 106.67	G - 0.301	4.37×10 ⁻⁸	N/A	N/A	1.13 (1.07-1.18)*	SLE, RA	<i>PRDMI</i>
15	rs415890	6q27	167.26 - 167.47	C - 0.522	2.51×10 ⁻¹²	N/A	N/A	1.17 (1.12-1.22)	RA, Graves	<i>CCR6</i>
16	rs1456896	7p12	50.22 - 50.34	T - 0.69	1.20×10 ⁻⁸	N/A	N/A	1.14 (1.09-1.20)	AD, SLE, MCV, ALL	<i>IKZF1</i> , <i>ZPBP</i> , <i>FIGL1</i>
17	rs4871611	8q24	126.54 - 126.65	A - 0.609	1.51×10 ⁻¹²	N/A	N/A	1.17 (1.12-1.23)	UC, Myelo.	<i>JAK2</i>
18	rs10758669	9p24	4.93 - 5.29	C - 0.349	1.00×10 ⁻¹³	N/A	N/A	1.18 (1.13-1.23)	UC, Lepr., SpA	<i>TNFSF15</i> , <i>TNFSF8</i>
19	rs3810936	9q32	116.47 - 116.74	C - 0.682	1.00×10 ⁻¹⁵	N/A	N/A	1.21 (1.15-1.27)	UC	<i>CREM</i> (6.4)
20	rs12242110	10p11	35.22 - 35.94	G - 0.315	1.10×10 ⁻⁹	N/A	N/A	1.15 (1.10-1.20)	BC	<i>ZNF365</i>
21	rs10761659	10q21	63.97 - 64.43	G - 0.538	4.37×10 ⁻²²	N/A	N/A	1.23 (1.18-1.29)	UC	<i>NKX2-3</i>
22	rs4409764	10q24	101.26 - 101.33	T - 0.492	2.29×10 ⁻²⁰	N/A	N/A	1.22 (1.17-1.27)	Atopy [‡]	<i>C1orf30</i>
23	rs7927997	11q13	75.70 - 76.04	T - 0.389	5.62×10 ⁻¹³	N/A	N/A	1.17 (1.12-1.22)		

No.	dbSNP ID	Chr.	Left - right (Mb)	Risk allele - Allele frequency in control population	P-value meta	P-value repl.	P comb.	OR (95% CI); *Loci with evidence of > than 1 independent association	Reported association	Positional candidate genes of interest; some additionally highlighted by ▲ 1KG eSNP(s) in LD; GRAIL (bold); eQTL (LOD score)
24	rs11564258	12q12	38.42 - 39.31	A - 0.025	6.17×10 ⁻²¹	N/A	N/A	1.74 (1.55-1.95)	PD, Lepr.	▲ <i>MUC19</i> , <i>LR6K2</i>
25	rs3764147	13q14	43.13 - 43.54	G - 0.245	1.41×10 ⁻¹⁰	N/A	N/A	1.17 (1.12-1.23)	Lepr.	▲ <i>CI3orf31</i>
26	rs2076756	16q12	49.02 - 49.41	G - 0.26	3.98×10 ⁻⁶⁹	N/A	N/A	1.53 (1.46-1.60)	Lepr., Atopy, Blau, GvHD	<i>NOD2</i>
27	rs2872507	17q21	34.62 - 35.51	A - 0.458	1.51×10 ⁻⁹	N/A	N/A	1.14 (1.09-1.19)	Asthma, UC, PBC, T1D, RA, WBC	▲ <i>GSM1</i> , ▲ <i>ZBP2</i> , <i>ORMDL3</i> (20.3) <i>IKZF3</i>
28	rs11871801	17q21	37.57 - 38.25	A - 0.756	2.51×10 ⁻⁸	N/A	N/A	1.15 (1.10-1.21)	MS [↓] , obesity, HIES	▲ <i>MLX</i> , <i>STAT3</i>
29	rs1893217	18p11	12.73 - 12.92	G - 0.153	1.29×10 ⁻¹⁴	N/A	N/A	1.25 (1.18-1.32)	T1D [↓] , celiac	<i>PTPN2</i>
30	rs740495	19p13	1.04 - 1.13	G - 0.247	8.13×10 ⁻¹²	N/A	N/A	1.16 (1.10-1.21)	UC	<i>GPX4</i> , <i>SBN2</i>
31	rs1736020	21q21	15.62 - 15.77	C - 0.579	9.33×10 ⁻¹²	N/A	N/A	1.16 (1.11-1.21)		
32	rs2838519	21q22	44.41 - 44.52	G - 0.391	2.09×10 ⁻¹⁴	N/A	N/A	1.18 (1.13-1.23)	Celiac, UC	<i>ICOSLG</i>

[†]*IL18RAP* and *CARD9* – association reported by Zhemakova *et al.*⁷ but not previously at genome-wide significance

[‡]loci previously reported at genome-wide significance in GWAS studies published subsequent to design of the current replication experiment^{5,6}

[§]loci that showed suggestive association and replication in Barrett *et al.*¹ but not previously at genome-wide significance

[↑]association in the opposite direction in different traits

We identified genes of interest based on a variety of *in silico* techniques (see text for more details) – identified as ▲ for coding SNPs identified from 1000 Genomes Project or HapMap in linkage disequilibrium with our most associated SNP (for dbSNP IDs see Supplementary Table 5), bold text for GRAIL connectivity and underlined text for presence of an eQTL effect with LOD 5.0 (for details see Supplementary Results).

Loci tagged by rs4656940 and rs7554511 previously replicated strongly (0.00048 and 2.3×10⁻⁶ respectively in Barrett *et al.*¹) and still pass genome-wide significance on combined analysis.